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The Radula and Ascus of *Elysia chlorotica* Gould (Opisthobranchia: Ascoglossa)

by

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Abstract. The radulas from 230 *Elysia chlorotica* of body lengths 2–45 mm were examined. The ratio of the length of the tooth base to the length of the cusp was constant at 0.33. The number of teeth in the ascending and descending limbs of the radular ribbon was similar with means of 7.8 and 8.4. The ascus sac contained 6–76 discarded teeth. A literature report of 6 ascending limb teeth, 11 descending limb teeth, and 4 ascus teeth in a 20-mm specimen is inconsistent with the number of teeth observed in 20-mm specimens in this study, which had no less than 36 discarded teeth. The relationship of total number of teeth with body length varied (inexplicably) among years.

INTRODUCTION

THE ASCOGLOSSAN GASTROPODS are, with rare oophagous exceptions, specialists in algal sap-sucking (CLARK & BUSACCA, 1978) utilizing a single row of radular teeth to draw out the cell sap. The intertidal ascoglossan *Elysia chlorotica* Gould, 1870, which occurs from the Minas Basin (BAILEY & BLEAKNEY, 1967) to Florida (MARCUS, 1980), begins feeding upon the alga *Vaucheria* immediately after metamorphosis (WEST *et al.*, 1984) and soon becomes a vivid green due to the algal chloroplasts, which it retains in its tissues in a functional state (GRAVES *et al.*, 1979). It must, therefore, develop a functional tooth at a body length of less than 1 mm.

The *Elysia chlorotica* population in the marshes of the Minas Basin, Nova Scotia, often produces individuals 35–45 mm in length (BLEAKNEY & MEYER, 1979) which is larger than other accounts of lengths of 20–30 mm (ABBOTT, 1974; GOSNER, 1978). MARCUS (1972) reported *Elysia chlorotica* to possess 6 teeth in the ascending limb (where they are formed), 11 teeth in the descending limb, and 4 discarded teeth in the ascus sac. As ascoglossans continually produce teeth and store all discarded teeth in the ascus sac, we began this study to determine whether the size of the buccal mass and radula, and the number of radular teeth, would increase with increasing body length and if the ascus progressively enlarged to accommodate the many more discarded teeth of larger individuals.

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MATERIALS AND METHODS

Specimens of *Elysia chlorotica* have been collected from local salt marshes since 1965 and some of this material through to 1983 was used in this study. Material for the statistical analyses was collected in 1981 except for comparisons of tooth cusp versus tooth base, tooth length versus body length, and teeth number versus body length, which utilized 1981 and 1983 specimens. The 1981 sample comprised 85 specimens. An additional 145 specimens from 1966, 1970, 1973, 1981, 1982, and 1983 were also examined.

Specimens were measured while crawling at full extension in seawater, then relaxed in 1% urethane and preserved by transferring to Boardman's Solution for 15 min and finally storing in 70% ethanol. The radular material was prepared by either removing the entire head of small specimens or dissecting out the buccal mass (Figure 1) of larger animals. The radular material of the 1981 specimens was mounted in Berlese's Fluid (GASCOIGNE, 1975) and the teeth measured after the tissue cleared. As it was difficult to flatten the ascus and spread out the jumble of teeth, the Berlese's Fluid was removed by soaking in water overnight and then a tissue solubilizer, 0.5 N quaternary ammonium hydroxide (BLEAKNEY, 1982), was added to dissolve the tissues. The entire examining process was shortened to 20–30 min for specimens of other years by simply using the solubilizer as the clearing agent and the dissociation agent. Specimens were placed in depression

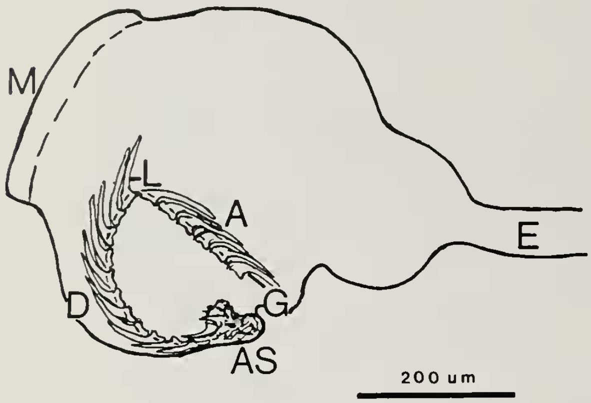

Figure 1

Buccal mass of *Elysia chlorotica* showing relative positions of ascending limb (A), ascus sac (AS), descending limb (D), esophagus (E), ghost tooth (G), leading tooth (L), and mouth (M).

slides and flooded with the solubilizer. The tissues cleared in approximately 4 min at which time the teeth in the ascending and the descending limbs were measured and counted. After an additional 10 min, the tissue was sufficiently digested to press beneath a coverslip and then the ascus contents were examined.

Two technique problems arose. First, many of the specimens over 15 mm body length had a red pigmented deposit in the ascus which adhered to and obscured the teeth. The tissue solubilizer had little effect, but the standard histological destaining solution of 1% acid-alcohol cleared the material overnight. Inexplicably, this was only noticed in individuals from the November 1965, August 1966, September–November 1981, and April–May 1983 samples.

Second, in specimens from the September 1981, April–May 1982, and October–November 1982 samples, the proximal part of the tooth shaft, where it attached to the larger rectangular tooth base, was tinted with a reddish pigment; invariably the tissue solubilizer digested this section of the tooth, immediately separating the shaft and base (Figure 2). Teeth in the ascending limb were unpigmented and unaffected. Accurate tooth counts for the descending limb and the ascus sac were obtained utilizing the solid rectangular tooth bases, as these are optically the most conspicuous portion of the tooth. These differences in solubility of the tissues and the pigment deposits were not related to body size nor season. Only teeth with red-tinted bases separated into two pieces, even after they had been in preservatives for over a year. Specimens from other years lacked the red pigment.

Radulas were prepared for scanning electron microscope (SEM) examination by dissolving the soft tissues with the solubilizer, then rinsing the solubilizer off with a drop of toluene. A drop of 95% ethanol was added and allowed to evaporate, which caused the radular ribbon to curl, thus exposing the tooth cusps. The radula was then pressed lightly into the smooth but tacky surface of a drop of black dissecting tray wax on an aluminum SEM stub. The final mount was coated with gold and palladium and examined using a JEOL JSM-255 scanning electron microscope.

RESULTS

Tooth length increased rapidly relative to body length for specimens of 5–20 mm length, then showed little increase for larger specimens even to body lengths of 40–45 mm: tooth length = 65.2 log(body length) + 15.8, $r = 0.903$, $P < 0.001$ (Figure 3). For most specimens, the newly generated teeth were the largest, and in the smallest individuals there was a graded series of these teeth along the radular ribbon (Figure 4). For specimens >10–15 mm body length, the teeth of the ascending radular limb were all about maximum size and there was only a slight or nil gradation evident (Figure 2). The magnitude of the initial gradation was conveniently recorded in the teeth of the ascus sac (see Figures 4, 5). However, unknown factors can affect this process of tooth generation, as we have found examples of a single tooth in a series with a base that was only one-half to two-thirds that of adjacent teeth. We also have found series of three to four new teeth being produced in decreasing sizes as in Figure 6 where the smallest tooth base was only 42% that of the largest tooth.

For teeth with cusp lengths of 40–120 μm, the relationship of base length to cusp length was linear: base = 0.33 cusp − 0.76, $P < 0.001$ (Figure 7). The regression line in Figure 7 may not be accurate for teeth with cusp lengths <25–30 μm, as the smallest teeth in the ascus generally were observed to have a cusp only as long as

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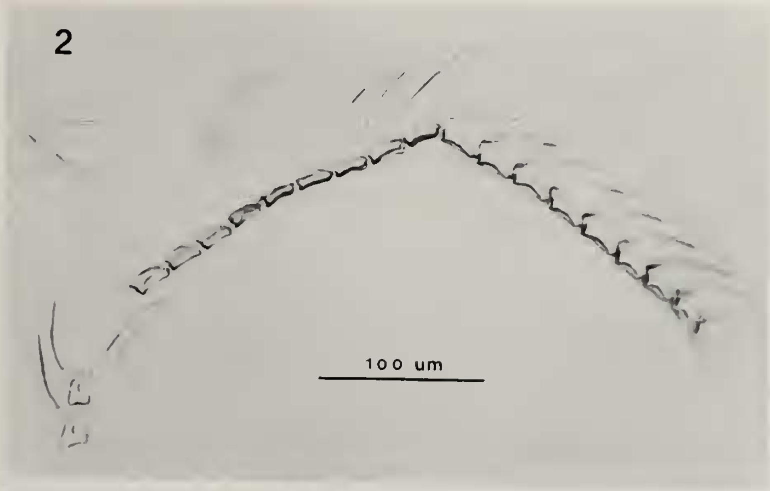
Explanation of Figures 2 and 4 to 6

Figure 2. Radula of *Elysia chlorotica* showing the differential effect of the tissue solubilizer on the bases of teeth from the ascending limb (at the right) and the descending limb (to the left). Ghost tooth is at extreme right and the two teeth at the lower left are in the transit tube to the ascus sac.

Figure 4. Part of a graded series of 31 teeth from the ascus sac of an *Elysia chlorotica* 4 mm in length.

Figure 5. Distribution of 64 teeth from an *Elysia chlorotica* 4 mm in length: ascending limb, $n = 8$; descending limb, $n = 8$; in transit tube, $n = 6$; in ascus sac, $n = 42$.

Figure 6. Ascending limb of a radula from an *Elysia chlorotica* (2 mm in length) with a large ghost tooth (at left) following an unusual series of four recently produced teeth that progressively decreased in size.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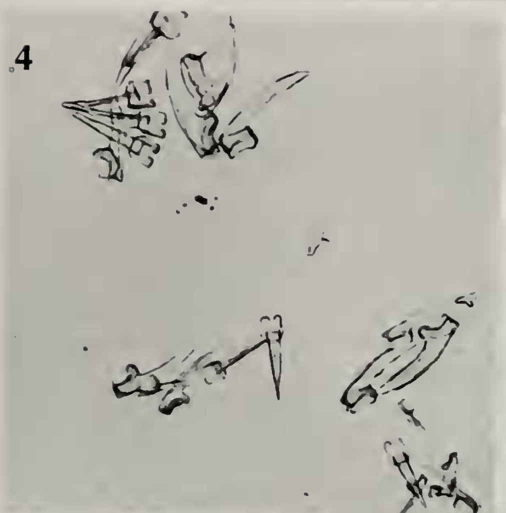
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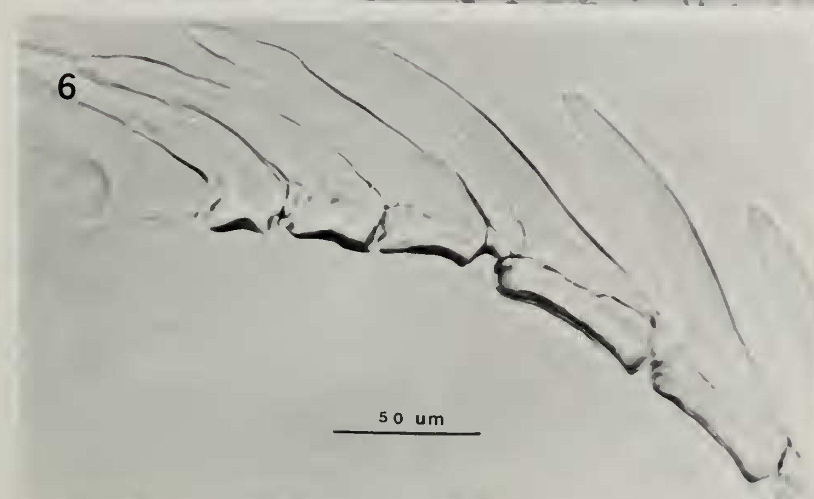
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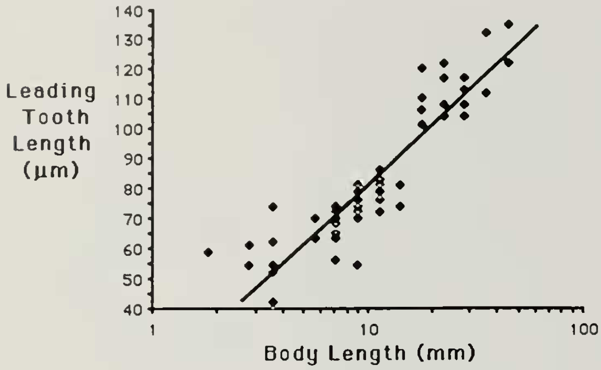

Figure 3

Leading tooth length versus body length of *Elysia chlorotica* collected from September 1981 to January 1982.

their 8-μm base whereas the regression line would indicate a cusp of approximately 24 μm for a tooth with an 8-μm base. Thus, a different development pattern was evident for these small, first-formed teeth.

The number of teeth in the ascending and descending limbs remained constant with no significant differences relative to body length (*F*-test, $P > 0.05$). In specimens from 2 to 46-mm body length, the ascending limb had 5–12 teeth (mean 7.8) and the descending limb had 5–13 teeth (mean 8.4). Additional teeth in the descending limb were often due to the radular ribbon remaining intact and extending into the tubular conduit between the descending limb and the ascus chamber (Figure 1).

The number of teeth stored within the ascus ranged from 6 to 76, with larger specimens having higher numbers. We have not examined any individuals less than 2 mm body length, but seven specimens (2–3 mm body length) averaged 23 teeth in the ascus sac and none had fewer than six. The highest tooth count was 76 in an ascus from a specimen 20 mm in body length, but there were seven larger animals (23–35 mm body length) in that same collection of 14 July 1983 that had ascus sacs so large and complex that they could not be dissected intact from the general connective tissue and thus these counts were lost. If an ascus sac or ascus complex were ruptured during dissection, several to many of the teeth would slip out and drift away under the coverslip, nullifying the possibility of a valid count. Those seven specimens may have had counts of 80–90 teeth within the ascus complex.

The ascus of *Elysia chlorotica* usually was a simple sac but was sometimes bilobed or even trilobed. The lobes, which were globose or elongate, were either clustered together at their bases or at the end of a narrow neck, or strung out under the esophagus in the form of a chain of two or three cylindrical chambers. The ascus often projected to either side of the buccal mass and sometimes was as long as the buccal mass and extended posteriorly under the esophagus. A tubular passageway extending from the descending limb to the ascus sac often contained several teeth that were detached from the radular ribbon and not yet compacted into the ascus chamber (two in Figure 2 and six in Figure 5). Teeth within the ascus were generally a jumbled mass of individual teeth, but occasionally there were linear series of 2–6 teeth arranged as they were on the radular ribbon. A coil of the first 8–10 teeth was found in the ascus sac on several occasions.

The total number of teeth, which is a function of the storage of discarded teeth within the ascus, varied tremendously and ranged from 6 to 76 teeth in our sample of 168 animals over the period 1966–1983. In large samples, the number of teeth increased with body length (Figure 8). In 1983, the number of teeth was higher in specimens collected during the summer months; ANCOVA, $F = 110.5$, $P = 0.001$ (Figure 9).

A single row of fine denticles, first photographed by BLEAKNEY (1982), extended from the tip of the cusp to

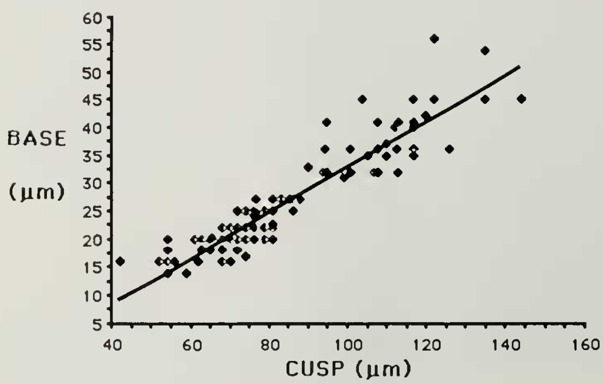

Figure 7

Tooth cusp length versus base length of various sizes of teeth from *Elysia chlorotica*.

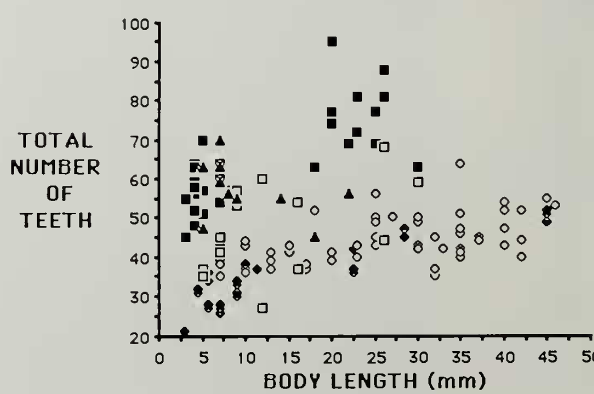

Figure 8

Total number of teeth versus body length of *Elysia chlorotica* from different samples. (▲—June, July 1970; ◆—September 1981; ◇—October, November 1982; ■—June, July 1983; □—October, November 1983)

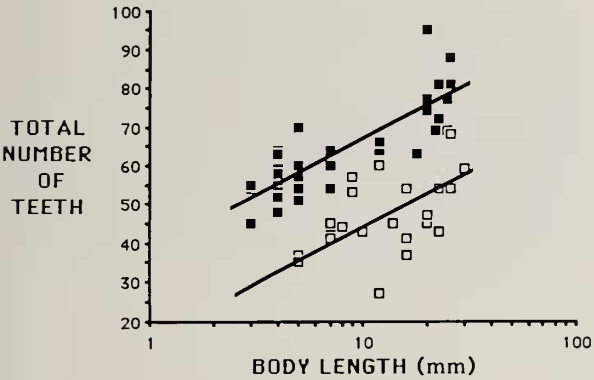

Figure 9

Total number of teeth versus body length of *Elysia chlorotica* from different seasons in 1983. (■—summer; □—fall)

the base on all teeth examined. The row of denticles was medial along the cusp, then curved to the right and terminated on the right shoulder of the basal plate, which was also the right side of the animal.

DISCUSSION

Most of the teeth of *Elysia chlorotica* have a similar morphology that is independent of the size of the tooth. Each tooth has a single row of minute denticles, which presumably assist in slicing open algae during feeding (BLEAKNEY, 1982). The ratio of base length to cusp length is constant at 0.33 (smallest measured had a cusp of 20 μm) except the first formed teeth, which have cusps that are not much larger than their base.

The numbers of teeth in the ascending and descending limbs are similar, with means of 7.8 and 8.4. There is no significant correlation between numbers of teeth in either limb with body length. The asci contained 6–76 teeth, but this may not be the full range as recently metamorphosed juveniles would not be expected to have teeth in the ascus, and the teeth of specimens with more than 70–80 teeth in the ascus could not be accurately counted. MARCUS' report (1972) of four teeth in the ascus of a 20-mm long *Elysia chlorotica* is not consistent with the present study because none of our 18–22-mm specimens had less than 36 teeth in the ascus.

The ascoglossan ascus is an enigmatic structure: why should a mollusk retain all its discarded teeth intact within a sac that has no obvious function? In *Elysia chlorotica* the teeth are evidently not broken down and recycled because a graded series of teeth is always present and the tissue solubilizer has no more effect on the old small teeth than on the new large ones. As more teeth are produced, the storage problem increases as is evident by configurations of the ascus in our samples. Although the ascus is usually considered as a simple sac projecting caudad from beneath the muscular buccal mass (GASCOIGNE & SARTORY, 1974), the ascus of *E. chlorotica* may also be bilobed or even trilobed.

In large samples, total number of teeth increased rapidly with body length among animals with body lengths up to approximately 10 mm, then slowed dramatically even though individuals reached 45 mm in length. Nevertheless, the description and analysis of the ascus tooth counts became more inexplicable sample by sample. Among different samples, similar-size animals can have dissimilar numbers of teeth; for example, ascus counts of 5-mm specimens ranged from 6 to 71 teeth.

The data from 1983 show two clusters of results (Figure 9). The highest counts were in June and July when tooth number was almost doubled for all size classes (Figures 8, 9). (The data from other years were inadequate to determine whether this was an annual occurrence. As well, insufficient knowledge of the natural growth of *Elysia chlorotica* prevented analysis of tooth number versus age.) The decrease in October and November of 1983 for specimens of similar size is unexpected because smaller individuals of that high-count population of July should have had even higher counts by autumn. These results may have been caused by an increase in body length without a corresponding increase in tooth number. Alternatively, if they voided some teeth and the ascus is not a total accumulation, the ascus would not contain the graded set of tooth sizes that was observed in all specimens. If the summer population died out, any new generation would have had to grow to 30–40 mm by mid-October. This is inconsistent with laboratory studies at 18–19°C where *E. chlorotica* was less than 10 mm in length at 73 days postmetamorphosis (WEST *et al.*, 1984).

Our analysis of the ascus contents became more inexplicable sample by sample and certainly contradicted our intuitive assumptions. We have recounted this experience so that other malacologists may be wary of ascoglossans bearing statistical gifts in the guise of a radular data bank.

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